Simultaneous determination and pharmacokinetics of five rhubarb anthraquinones in dog plasma by HPLC after orally administration the rhubarb extract

Su-xiang Feng[1,2], Jian-sheng Li[3]*, Shu-mei Wang[4], Ran Yang[1], Ti-qiang Zhou[3], Xiao-yu Li[2] and Ling-bo Qu[1]**

[1]College of Chemistry and Molecular Engineering, Zhengzhou University, Zhengzhou, China
[2]College of Pharmacy, Henan University of Traditional Chinese Medicine, Zhengzhou, China
[3]Institute of Geriatrics, Henan University of Traditional Chinese Medicine, Zhengzhou, China
[4]School of Traditional Chinese Materia Medica, Guangdong Pharmaceutical University, China

Abstract: Rhubarb is widely used in the treatment of obstipation, gastrointestinal indigestion and other diseases in China and other Asian countries for thousands of years. Anthraquinones are the major group of polyphenol constituents including aloe-emodin, rhein, emodin, chrysophanol and physcion. In order to evaluate the pharmacokinetics of five rhubarb anthraquinones, a high-performance liquid chromatography with fluorescence detection (HPLC-FLD) method for simultaneous determination of aloe-emodin, rhein, emodin, chrysophanol and physcion in dog plasma was established. Solid phase extraction (SPE) was applied to the extraction and purification of samples. The calibration curves of five anthraquinones showed good linearity with r greater than 0.9925. The average extraction recoveries, examined at three concentration levels, carried from 92.1% to 102.3%, and the accuracies ranged from 87.7% to 102.5% with precision (RSD) <10%. The pharmacokinetic paremeters of five anthraquinones were investigated systematically after orally administration the rhubarb extract. Five anthraquinones were rapidly absorbed and T_{max} for aloe-emodin, rhein, emodin, chrysophanol and physcion was at 0.75, 1.50, 0.75, 1.0 and 2.0 h respectively. The C_{max} of five anthraquinones was 0.031, 3.39, 0.27, 0.036 and 0.032 μg/mL while the AUC of five anthraquinones was 0.35 ± 0.058, 32.22 ± 8.29, 2.97 ± 0.66, 0.43 ± 0.10 and 0.41 ± 0.12 mg h/L, respectively.

Keywords: Anthraquinone; HPLC; dog plasma; pharmacokinetics, rhubarb extract.

INTRODUCTION

Rhubarb is dried *Rhei Radix et Rhizome of Rheum palmatum L., Rheum tanguticum Maxim. ex Balf., or Rheum officinale Baill* (National Commission of Chinese Pharmacopoeia, 2010). It has been widely used in the treatment of obstipation, gastrointestinal indigestion, diarrhea and jaundices in China and other Asian countries for thousands of years (Tang and Eisenbrand 1992; Newall *et al.*, 1996). The main active components in rhubarb are aloe-emodin, rhein, emodin, chrysophanol, physcion (structures shown in fig. 1) and their glucosides. Numerous studies reported that the anthraquinones exhibited various bioactivities such as antifungal (Agarwal *et al.,* 2000), antimicrobial (Hsiang and Ho, 2008), antitumour (Koyamaa *et al.*, 2002, Su *et al.*, 2005, Huang *et al.,* 2007), antioxidant (He *et al.,* 2009, Iizuka *et al.,* 2004, Kim *et al.,* 2010, Hsu *et al.,* 2007) and antihuman cytomegalovirus activity (He *et al.,* 2010). In recent years, a great amount of works has been done on rhubarb anthraquinones (Li *et al.,* 2005, Li *et al.,* 2010, Li *et al.,* 2011, Liu *et al.,* 2010). The found that rhubarb anthraquinones possessed of protective effects against focal cerebral ischemia by decreasing plasminogen and fibrinogen, prolonging blood coagulation or resisting the aggregation and adhesion of platelet. Therefore, it is necessary to monitor the plasma pharmacokinetics of five rhubarb anthraquinones in animals.

Various bioanalytical methods have been reported for the quantification of rhubarb anthraquinones including high-performance liquid chromatography coupled with ultraviolet detection (Tang *et al.,* 2007, Shia *et al.,* 2011, Ding, *et al.,* 2003, Shia *et al.,* 2009) and fluorescence detection (Yan *et al.,* 2007, Zaffaroni *et al.,* 2003). HPLC methods coupled with mass spectrometry (HPLC-MS) (Layek *et al.,* 2008) for quantitation of rhein in human plasma has also been published previously. Although HPLC-MS offers excellent selectivity and sensitivity and shorter analysis time than HPLC methods, it requires relatively expensive instrumentation and highly skilled technical expertise. Moreover, anthraquinones display strong fluorescence properties and thus more readily detected using a fluorescence detector without the need for lengthy derivatization procedures. In addition, the previous researches mostly concentrated on the pharmacokinetic studies of parts of rhubarb anthraquinones. As the pharmacological actions of

*Corresponding author: e-mail: li_js8@163.com, shmwang@sina.com

rhubarb compounds were attributable to a synergistic effect of multiple components and therefore it is of great importance to the determination of multiple components of rhubarb entering into the body for evaluating the efficacy and investigating the action mechanism. Furthermore, although much attention had been paid to the pharmacokinetics of anthraquinones in rats or mice, no information on the pharmacokinetic study of five rhubarb anthraquinones in dogs had been reported in detail.

The aim of this study was to establish a sensitive HPLC-FLD method for the quantification of aloe-emodin, rhein, emodin, chrysophanol, physcion in dog plasma. SPE was applied to extract and purify of five analytes in biological samples. The reliable SPE-HPLC-FLD method showed a high sensitivity and good resolution and was suitable for the determination of rhubarb anthraquinones in dog plasmas. The validated method was successfully applied to the pharmacokinetic studies of five rhubarb anthraquinones in beagle dogs after oral administration of rhubarb extract.

MATERIALS AND METHODS

Chemicals and reagents

The reference standards of aloe-emodin, rhein, emodin, chrysophanol, physcion and 1, 8-dihydroxyanthraquinone (internal standard, IS) were all purchased from the National Institutes for Food and Drug Control (purity >98%) (Beijing, China). SPE cartridges (C_{18}, 3 mL, packed with 200 mg of 50 μm particle sizes) were purchased from Bonna-Agela Technologies (Tianjin, China). HPLC grade methanol was purchased from Dikma Technology Inc. (Beijing, China). Purified water was obtained from a Millipore Milli-Q system (Bedford, MA, USA). All other reagents used in this study were all of analytical grade. Rhubarb extract was prepared in our laboratory. The contents of aloe-emodin, rhein, emodin, chrysophanol and physcion in the rhubarb extract were 87, 115, 231, 263 and 176 mg/g, respectively.

Chromatographic conditions

The HPLC system (Waters, USA) consists of 2695 separation module, 2475 fluorescence detector and Empower 2 chromatography analytical workstation. The chromatographic separation was performed on a Venusil XBP C_{18}(L) column (4.6 × 250 mm, 5 μm, Bonna-Agela Technologies, Tianjin, China) protected by a Waters security guard C18 column (4.6 × 20 mm, 5 μm). The mobile phase consisted of a mixture of methanol-0.1% phosphoric acid water (75:25, v/v) at a flow rate of 1.0 mL/min. Column temperature was maintained at 30°C. The column eluent was monitored with a fluorescence detector at wavelengths of 435 nm and 515 nm for excitation and emission, respectively.

Preparation of plasma sample

The solid-phase extraction method was used for extraction and pre-concentration of anthraquinones from dog plasma. SPE cartridges were equilibrated with 2 mL methanol and 2 mL purified water. 200 μL plasma, 25 μL IS and 2 mL methanol was vortexed quickly for 5 min and then centrifuged at 12,000 rpm for 15 min at 4 ˚C (Refrigerated Centrifuge 3-18k, Sigma, German). As finished, the supernatant was collected with a clean tube carefully and diluted with water containing 0.1% phosphoric acid. Then the mixture was loaded into the cartridge. After washing the SPE column with 2 mL purified water, the analytes were eluted with 2 mL methanol. Afterward, the eluate was evaporated to dryness under a stream of nitrogen at room temperature. Finally, the residue was dissolved in 100 μL methanol and 20 μL of the mixture was injected into HPLC system for analysis.

Preparation of standards and quality control solutions

Stock solutions of aloe-emodin, rhein, emodin, chrysophanol, physcion and 1, 8-dihydroxyanthraquinone (IS) were prepared in methanol separately at concentrations of 5.55, 39.0, 7.35, 5.10, 5.90 and 1.6 μg/mL.

The quality control (QC) samples were prepared at high, medium and low concentrations of five anthraquinones. The concentrations were 11.4, 28.4 and 71.0 ng/mL for aloe-emodin, 31.9, 1248 and 3900 ng/mL for rhein, 37.6, 94.1 and 588.0 ng/mL for emodin, 10.4, 65.3 and 163.2 ng/mL for chrysophanol and 12.1, 30.2 and 188.8 ng/mL for physcion with six replicates for each concentration. All plasma samples were processed according to the procedure described above.

Method validation

Selectivity

The selectivity of the method was evaluated by comparing the chromatograms of blank plasma, standard sample, spiked plasma and plasma after oral administration of rhubarb extract.

Linearity and sensitivity

Calibration standards of the mixture anthraquinones were prepared by spiking the standard mixture working solutions into 200 μL blank plasma in a tube containing 25 μL I.S. The linearity of calibration curve was prepared by plotting the peak area ratio versus the concentration of five anthraquinones. The calibration curves of aloe-emodin, rhein, emodin, chrysophanol and physcion were linear over the range of 11.4-1110, 31.9-7800, 37.6-1470, 10.4-1020, and 12.1-1180 ng/mL, respectively. The limit of quantification (LOQ) was established as the lowest concentration on the calibration curve which can be measured with acceptable precision and accuracy. The

Table 1: Intra-day and inter-day accuracy and precision of anthraquinones in dog plasma (n =five days and six replicates per day)

Anthraquinones	Spiked concentration (ng/mL)	Intra-day			Inter-day		
		Measured concentration (ng/mL, mean ± SD)	Accuracy (%)	RSD (%)	Measured concentration (ng/mL, mean ± SD)	Accuracy (%)	RSD (%)
Aloe-emodin	11.4	10.0 ± 0.68	87.7	6.81	10.1 ± 0.64	88.7	6.36
	28.4	26.4 ± 1.53	93.0	5.77	26.5 ± 1.50	93.3	5.67
	71.0	67.7 ± 3.46	95.3	5.11	64.4 ±3.33	90.6	5.17
Rhein	31.9	31.2 ±2.01	97.9	6.45	30.0 ± 2.04	94.2	6.78
	1248	1231.9 ± 54.1	98.7	4.39	1267.4 ± 58.7	101.6	4.63
	3900	3950.2 ± 195.8	101.3	4.96	3997.1 ± 148.7	102.5	3.72
Emodin	37.6	35.3±1.91	94.0	5.40	35.4 ± 2.06	94.1	5.81
	94.1	90.2 ± 5.75	95.8	6.38	89.3 ± 5.53	94.9	6.19
	588.0	560.0 ± 26.7	95.2	4.77	559.3 ± 32.0	95.1	5.73
Chrysophanol	10.4	9.9 ± 0.71	95.2	7.17	9.7 ± 0.73	93.4	7.49
	65.3	63.1 ± 3.54	96.6	5.62	64.1 ± 4.14	98.2	6.45
	163.2	158.6 ± 7.98	97.2	5.03	157.3 ± 8.29	96.4	5.27
Physcion	12.1	10.7 ± 0.83	88.3	7.80	11.4 ± 0.92	93.9	8.06
	30.2	29.2 ± 1.88	96.6	6.44	28.5± 1.96	94.3	6.90
	188.8	178.6 ±8.31	94.6	4.65	184.6± 10.0	97.8	5.44

Table 2: Stability of five anthraquinones in dog plasma under indicated storage conditions (mean ± SD, n = 6).

Anthraquinones	Spiked concentration (ng/mL)	Three freeze-thaw cycles	Storage at -20°C to 30 days	Auto-sampler 4°C for 12 h
Aloe-emodin	11.4	93.2± 7.12	91.6 ± 6.71	93.8±5.72
	28.4	97.6± 4.82	95.3± 8.02	97.4± 6.82
	71.0	96.8± 6.15	96.3 ± 8.32	96.9± 4.15
Rhein	31.9	95.4± 6.27	93.6 ± 9.05	96.1± 8.74
	1248	102.1± 8.03	98.5 ± 5.97	101.1± 6.83
	3900	98.3± 4.84	101.4 ± 4.43	99.2± 5.39
Emodin	37.6	94.2± 6.68	96.3 ±5.82	92.2± 4.18
	94.1	94.7± 5.08	97.2 ± 7.71	99.4± 7.29
	588.0	92.5± 5.27	96.1 ± 4.94	98.9± 5.97
Chrysophanol	10.4	95.4± 6.84	94.8 ± 7.70	95.2± 8.89
	65.3	98.6 ± 7.73	92.4 ± 6.86	94.7 ± 6.11
	163.2	95.8± 4.11	97.1 ±5.22	95.3± 4.94
Physcion	12.1	94.6± 5.18	92.8 ± 7.25	93.4± 8.38
	30.2	94.1± 4.23	91.6± 6.27	93.5± 5.92
	188.8	93.7± 6.98	95.2 ± 3.76	97.0± 7.72

limit of detection (LOD) was defined as the amount that could be detected with a signal-to-noise ratio of 3.

Precision and accuracy

To evaluate the precision and accuracy of the method, QC samples at three concentration levels were analyzed in six replicates on the same day and on five consecutive validation days, respectively. The results of the intra-day and inter-day precisions were presented with the relative standard deviation (RSD %), whilst percentage difference between amount spiked and determined was taken as the measures of accuracy.

Extraction recovery and stability

The recovery of five anthraquinones from plasma at three different concentrations was calculated by comparing peak area ratios with QC samples. It was evaluated by analyzing six replicates of each QC samples. The recovery of IS was evaluated in a similar way.

The stability of anthraquinones in dog plasma was evaluated under a variety of storage and handling conditions with the low, medium and high QC samples. Freeze-thaw stability was tested by conducting three freeze (−20°C)/thaw (room temperature) cycles on

Table 3: Pharmacokinetic parameters of five anthraquinones in beagle dogs after a single oral dose of the rhubarb extract (mean ± SD, n = 6).

Parameters	Aloe-emodin	Rhein	Emodin	Chrysophanol	Physcion
$AUC_{(0-t)}$ (mg h /L)	0.35 ± 0.058	32.22 ± 8.29	2.97 ± 0.66	0.43 ± 0.10	0.41 ± 0.12
$AUC_{(0-\infty)}$ (mg h /L)	0.42 ± 0.083	35.15 ± 10.23	4.05 ± 0.94	0.54 ± 0.14	0.48 ± 0.14
C_{max} (mg/L)	0.031 ± 0.005	3.39 ± 0.43	0.27 ± 0.061	0.036 ± 0.009	0.032 ± 0.006
T_{max} (h)	0.75 ± 0.11	1.50 ± 0.20	0.75 ± 0.16	1.00 ± 0.25	2.00 ± 0.26
$t_{1/2}$ (h)	14.73 ± 6.52	10.11 ± 2.29	18.73 ± 3.82	15.18 ± 2.92	13.08 ± 2.19
CL (L/h/kg)	61.63 ± 10.47	0.98 ± 0.22	17.12 ± 5.53	146.61 ± 47.18	109.53 ± 33.36
Vd (L/kg)	1309.86 ± 555.46	14.33 ± 3.03	462.72 ± 123.65	3210.97 ± 800.29	2067.15 ± 656.96

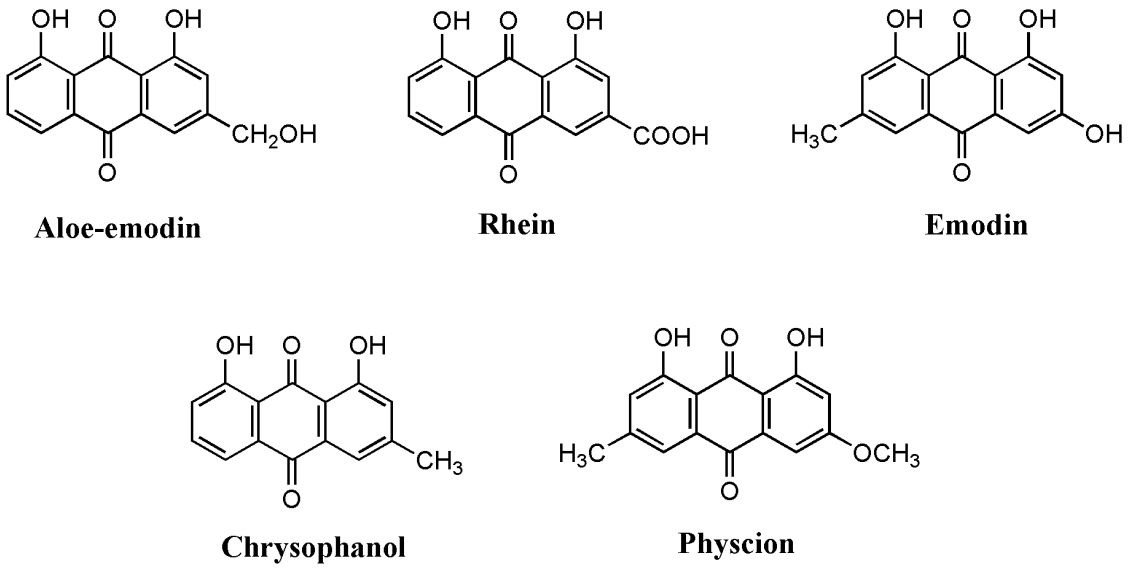

Fig. 1: The chemical structure of five anthraquinones.

consecutive days. The post-preparation stability was examined by determining the extracted QC samples stored in the auto-sampler (4°C) for 12 h. The storage stability was evaluated by using QC plasma samples stored at −20°C for 30 days. A stability/reference samples ratio of 85-115% was accepted as the stability criterion.

Pharmacokinetic study

Six male beagle dogs (7-8 months old, 7.2-10.5 kg) were purchased from the Laboratory Animal Center of Suzhou Xishan Zhongke Limited Company (Certificate No. SCXK2007-2008). Prior to administration, each dog was fasted with free access to water for 12 h and then orally administered rhubarb extract (150 mg/kg). Blood sample from the dog forelimb veins about 0.5 mL was drawn into a heparinized tube at the time point of 0, 0.083, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0 and 36.0 h after oral administration. All blood samples were immediately centrifuged and the supernatant was stored at −20°C until analysis. The study protocol was approved by the Institutional Animal Care and Use Committee of Zhengzhou University.

The main pharmacokinetic parameters, such as $t_{1/2}$ (the biological half-life), CL (total plasma clearance) and AUC (under the plasma concentration-time curve) were calculated by the non-compartmental method with the aid of the Drug and Statistics 2.0 (DAS2.0) (Mathematical Pharmacology Professional Committee of China, Shanghai, China). C_{max} (the peak plasma concentration) and T_{max} (the time to reach peak concentration) values were obtained directly from the observed concentration *vs* time data.

RESULTS

Selectivity

The representative chromatograms of five anthraquinones and IS are shown in fig. 2. The results indicated that the five anthraquinones and IS was not detected in blank plasma and could be well separated and detected in spiked plasma and plasma after oral administration of rhubarb extract.

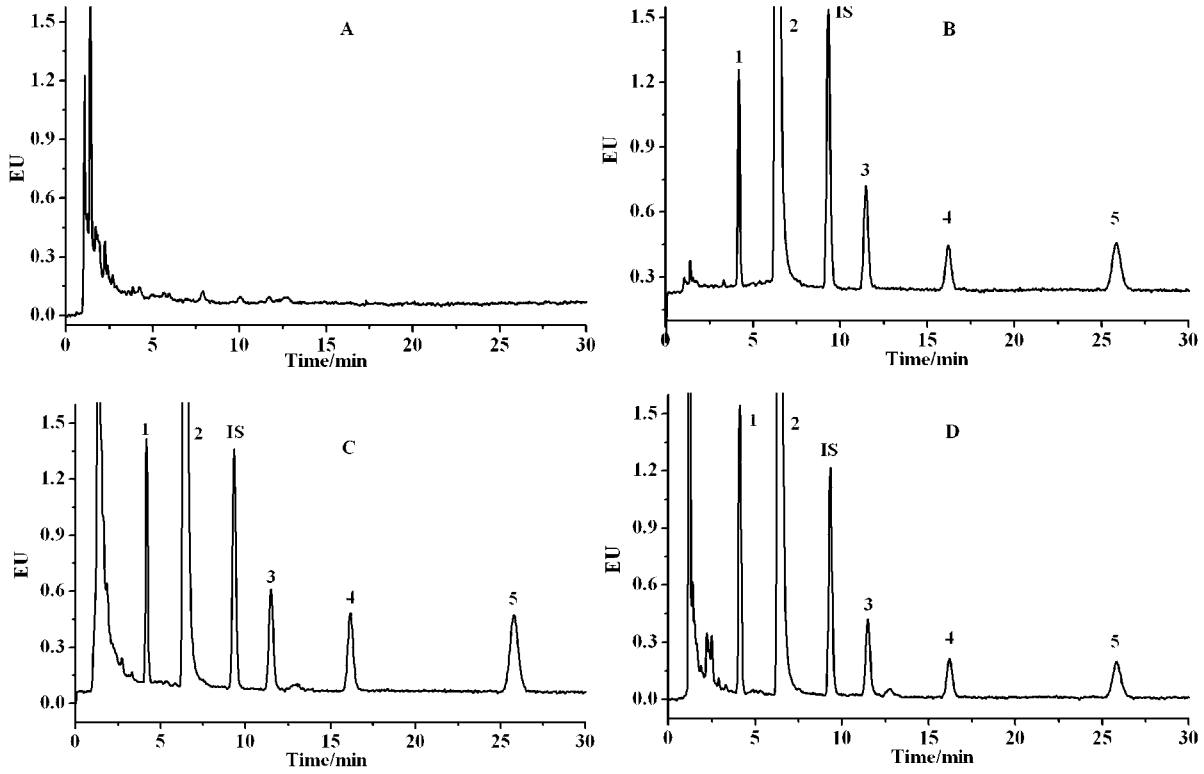

Fig. 2: HPLC Chromatograms of dog plasma samples: (A) blank plasma; (B) standard sample; (C) plasma spiked with aloe-emodin (1), rhein (2), internal standards (I.S.), emodin (3), chrysophanol (4) and physcion (5); (D) plasma sample collected at 1 h in beagle dogs after oral administration of rhubarb extract.

Linearity and sensitivity

To evaluate the linearity of the method established, the calibration curves were calculated plotting the peak area ratios against the analyte concentrations. The calibration curves were y = 0.2850 + 0.0021x (r=0.9976), y = 0.1289 + 0.0013 x (r=0.9991), y = 0.5964 + 0.0050x (r=0.9925), y = 0.2867 + 0.0028x (r=0.9952) and y = 0.2594 + 0.0016x (r=0.9938) in the content range for aloe-emodin, rhein, emodin, chrysophanol and physcion. The LOD are estimated to be 3.8, 10.6, 12.5, 3.5 and 4.0 ng/mL and the LOQ are 11.4, 31.9, 37.6, 10.4 and 12.1 ng/mL, respectively.

Precision and accuracy

Precision was evaluated as the relative standard deviation (RSD %) and the results are listed in table 1. The intra- and inter-day precision ranged 4.39-7.80 and 3.72-8.06, respectively. The accuracy derived from QC samples was between 87.7% and 102.5% for all the QC levels of five analytes. The results indicated that the precision and accuracy of the method was acceptable in dog plasma.

Recovery and stability

The mean recoveries of five anthraquinones in dog plasma at three different spiked concentration levels were found to be in the range of 92.1-97.1%, 95.2-102.3%, 94.4-97.7%, 93.1-95.2% and 93.4-96.0% for aloe-emodin, rhein, emodin, chrysophanol and physcion, respectively. The RSDs were less than 9.01%. The recovery of the IS was 95.7 %.

Table 2 indicated that the accuracies of five analytes in dog plasma were in the range of 91.6-102.1% for three freeze-thaw cycles, 12 h in the auto-sampler (4˚C) and 30 days at −20˚C. The results suggested that the anthraquinones were stable and no significant degradations were observed over the process under indicated storage conditions.

DISCUSSION

The proposed method was successfully applied to the pharmacokinetic study in beagle dogs after oral administration of rhubarb extract. The concentration-time profile of the five anthraquinones is illustrated in fig. 3 and the main pharmacokinetic parameters are presented in table 3.

As shown in fig. 3, five compounds were rapidly absorbed and the T_{max} peaked at 0.75, 1.50, 0.75, 1.0 and 2.0 h for aloe-emodin, rhein, emodin, chrysophanol and physcion respectively after oral dose of 150 mg/kg

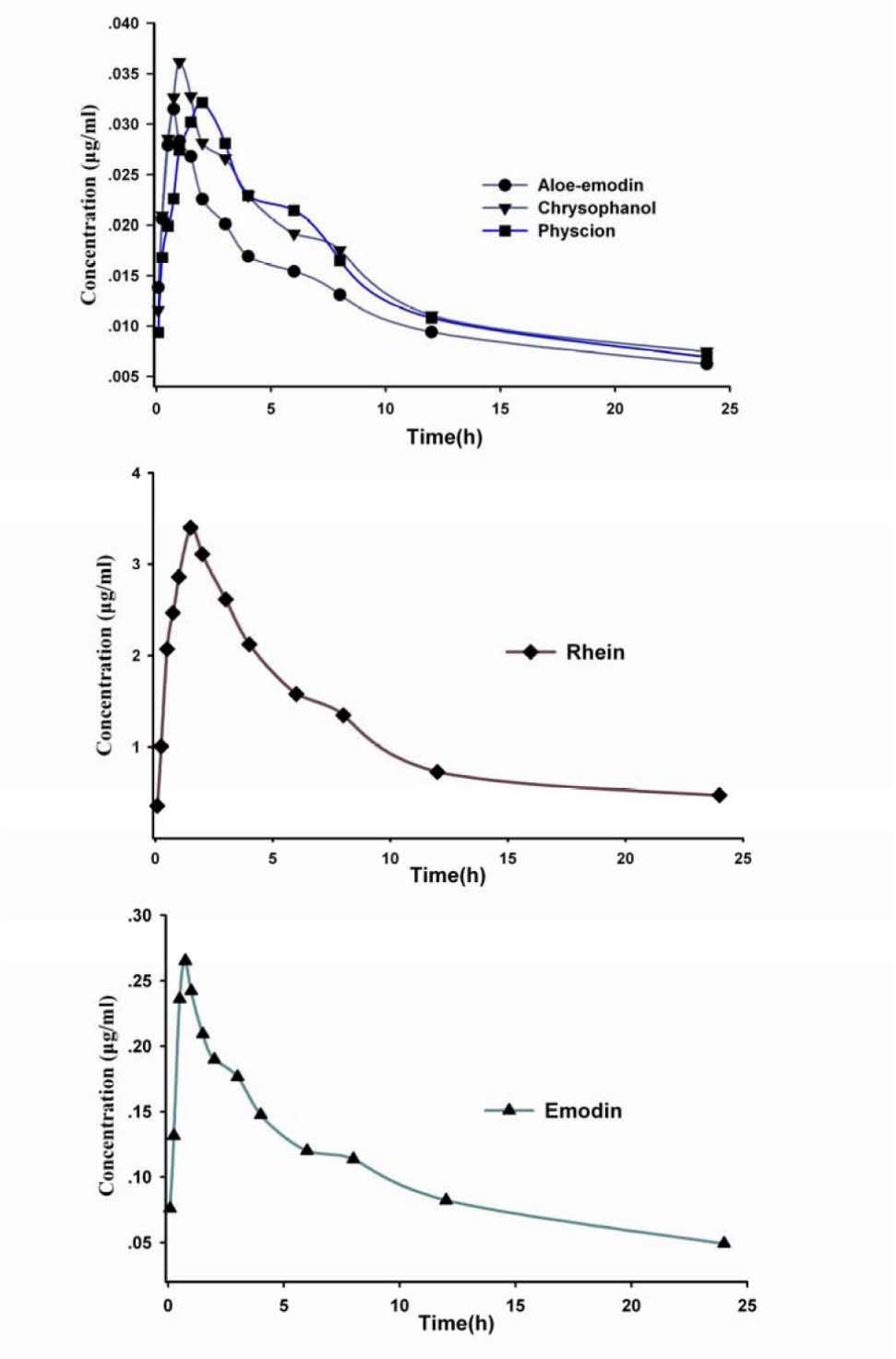

Fig. 3: Mean concentration–time profiles of five anthraquinones in beagle dogs.

rhubarb extract. The C_{max} was 0.031, 3.39, 0.27, 0.036 and 0.032 μg/mL while half-life ($t_{1/2}$) values were 14.73, 10.11, 18.73, 15.18 and 13.08 h for aloe-emodin, rhein, emodin, chrysophanol and physcion, respectively.

It can be seen from table 3 that the pharmacokinetic parameters such as T_{max}, C_{max}, $t_{1/2}$ and AUC of five anthraquinones were significant different. T_{max} of rhein and physcion was longer than those of aloe-emodin,

emodin and chrysophanol whereas $t_{1/2}$ of rhein was much shorter than other four components. For instance, $t_{1/2}$ of rhein was nearly a half of that of emodin and the clearance of rhein was much quick correspondingly.The peak concentration and AUC of rhein were more than 10 times higher than emodin and 100 times higher than aloe-emodin, chrysophanol and physcion. This implied that rhein might be the most important active compound absorbed in the body among five anthraquinones. These results are in accordance with the findings of previous studies (Yan *et al.,* 2009; Tang *et al.,* 2007). The unusual higher concentration of rhein may be accounted for the presence of aloe-emodin and chrysophanol, which could be oxidized and converted to rhein and rhein glucuronide/sulfate conjugates (Mueller *et al.,* 1998; Song *et al.,* 2009), and there is no evidence to show that rhein reduces to aloe-emodin or emodin(Krumbiegel and Schulz, 1993).

CONCLUSION

A selective and sensitive HPLC-FLD method was proposed to evaluate the pharmacokinetics of five rhubarb anthraquinones in dogs. SPE was employed for the extraction and purification of the five components from plasma samples. The method offered high extraction recoveries and good accuracy and precision and was suitable for biological samples analysis. Experimental results showed that rhein had the higher concentration in plasma than other four rhubarb anthraquinones which indicated rhein might be the most important active compound absorbed in the body among five rhubarb anthraquinones. The present pharmacokinetic study of five rhubarb anthraquinones in dogs will provide helpful information for the clinical applications.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (No.81274179, No.81073024) and Foundation for University Young Teachers by Henan Province (No.2009GGJS-065).

REFERENCES

Agarwal SK, Singh SS, Verma S and Kumar S (2000). Antifungal activity of anthraquinone derivatives from Rheum emodi. *J. Ethnopharmacol.,* **72**: 43-46.

Ding MY, Ma SW and Liu DL (2003). Simultaneous determination of hydroxyanthraquinones in rhubarb and experimental animal bodies by high-performance liquid chromatography. *Anal. Sci.,* **19**: 1163-1165.

He ZH, He MF, Ma SC, But PP (2009). Anti-angiogenic effects of rhubarb and its anthraquinone derivatives. *J. Ethnopharmacol.,* **121**: 313-317.

He ZH, Zhou R, He MF, Lau CB, Yue GG, Ge W and But PP (2010). Anti-angiogenic effect and mechanism of rhein from Rhizoma Rhei. *Phytomedicine*, **18**: 470-478.

Hsiang CY, Ho TY (2008). Emodin is a novel alkaline nuclease inhibitor that suppresses herpes simplex virus type 1 yields in cell cultures. *Brit. J. Pharmacol,,* **155**: 227-235.

Hsu CY, Chan YP and Chang J (2007). Chang, Antioxidant activity of extract from Polygonum cuspidatum. *Biol. Res.,* **40**: 13-21.

Huang Q, Lu G, Shen HM, Chung MC and Ong CN (2007). Anti-cancer properties of anthraquinones from rhubarb. *Med. Res. Rev.,* **27**: 609-630.

Iizuka A, Iijima OT, Kondo K, Itakura H, Yoshie F, Miyamoto H, Kubo M, Higuchi M, Takeda H and Matsumiya T (2004). Evaluation of Rhubarb using antioxidative activity as an index of pharmacological usefulness. *J. Ethnopharmacol.,* **91**: 89-94.

Kim J, Kim MY, Leem KH, Moon S, Jamakattel-Pandit N, Choi H, Kim H and Bu Y (2010). Key compound groups for the neuroprotective effect of roots of Polygonum cuspidatum on transient middle cerebral artery occlusion in Sprague-Dawley rats. *Nat. Prod. Res.,* **24**: 1214-1226.

Krumbiegel G and Schulz HU (1993). Rhein and aloe-emodin kinetics from senna laxatives in man. *Pharmacology*, **47**: 120-124.

Koyama J, Morita I, Tagahara K, Nobukuni Y, Mukainaka T, Kuchide M, Tokuda H and Nishino H (2002). Chemopreventive effects of emodin and cassiamin B in mouse skin carcinogenesis. *Cancer Lett,,* **182**: 135-139.

Layek B, Kumar TS, Trivedi RK, Mullangi R and Srinivas NR (2008). Development and validation of a sensitive LC-MS/MS method with electrospray ionization for quantitation of rhein in human plasma: application to a pharmacokinetic study. *Biomed. Chromatogr.,* **22**: 616-624.

Li JS, Wang D, Fang J, Zhang WY, Liu JX and Zhou HX (2005). Effect of different-dose rhubarb aglycone on thrombosis, platelet aggregation and adhesion, and blood coagulation in rats with cerebral ischemia: comparison with aspirin and Nimodipine. *China J Clin Rehabil*, **21**: 142-145.

Li JS, Liu JX, Wang D, Liu K, Liang SW, Sun J, Su J, Li N and Guo XY (2010). Effects of rhubarb aglycone combined with thrombolysis on brain m icrovascular basement membrane impairment in rats with thrombus-occluded cerebral ischemia. *China J Chin Mater Med.,* **35**: 2908-2911.

Li JS, Liu JX, Wang D, Liang SW, Liu K, Guo XY, Sun J, Li N and Su J (2011). Study of Rhubarb aglycone on time window of thrombolysis therapy through artery in rats with thrombus-occluded cerebral ischemia. *China J. Tradit. Chin. Med. Pharm,,* **26**: 1967-1971.

Liu JX, LI JS, Wang D, Liu K, Liang SW, Li N, Sun J, Guo XY and Su J (2010). Influence of Rhubarb aglycone associated thrombolysis on neurocyte Apoptosis in Rats with thrombus-occluded cerebral

ischemia. *Chin. J. Exp. Tradit. Med. Form,,* **16**: 119-123.

Mueller SO, Stopper H and Dekant W (1998). Biotransformation of the anthraquinones emodin and chrysophanol by cytochrome P450 enzymes. *Drug Metab. Dispos.,* **26**: 540-546.

National Commission of Chinese Pharmacopoeia, Pharmacopoeia of the People's Republic of China (2010). China Medical Science Press, Beijing, China, p.22.

Newall CA, Anderson LA and Phillipson JD (1996). *Herbal Medicines.* The Pharmaceutical Press: London, United Kingdom, p.228.

Shia CS, Juang SH, Tsai SY, Chang PH, Kuo SC, Hou YC and Lee Chao PD (2009). Metabolism and pharmacokinetics of anthraquinones in rheum palmatum in rats and *ex vivo* antioxidant activity. *Planta. Med.,* **75**: 1386-1392.

Shia CS, Tsai SY, Lin JC, Li ML, Ko MH, Lee Chao PD, Huang YC and Hou YC (2011). Steady-state pharmacokinetics and tissue distribution of anthraquinones of Rhei Rhizoma in rats. *J. Ethnopharmacol.,* **137**: 1388-1394.

Song R, Lin H, Zhang Z, Li Z, Xu L, Dong H and Tian Y (2009). Profiling the metabolic differences of anthraquinone derivatives using liquid chromatography/tandem mass spectrometry with data-dependent acquisition. *Rapid Commun. Mass Spectrom.*, **23**: 537-547.

Su YT, Chang HL, Shyue SK and Hsu SL (2005). Emodin induces apoptosis in human lung adenocarcinoma cells through a reactive oxygen species-dependent mitochondrial signaling pathway. *Biochem. Pharmacol.,* **70**: 229-241.

Tang W and Eisenbrand G (1992). Chinese Drugs of Plant Origin, Springer-Verlag: Berlin, Heidelberg, Germany, p.855.

Tang WF, Huang X, Yu Q, Qin F, Wan MH, Wang YG and Liang MZ (2007). Determination and pharmacokinetic comparison of rhein in rats after oral dosed with Da-Cheng-Qi decoction and Xiao-Cheng-Qi decoction. *Biomed. Chromatogr.,* **21**: 1186-1190.

Yan DM and Ma YM (2007). Simultaneous quantification of five anthraquinones in rat plasma by high-performance liquid chromatography with fluorescence detection. *Biomed. Chromatogr.,* **21**: 502-507.

Yan DM, Ma YM, Shi R, Xu DS and Zhang N (2009). Pharmacokinetics of anthraquinones in Xiexin decoction and in different combinations of its constituent herbs. *Phytother. Res.,* **23**: 317-323.

Zaffaroni M, Mucignat C, Pecere T, Zagotto G, Frapolli R, D'lncalci M and Zucchetti M (2003). High-performance liquid chromatographic assay for the determination of Aloe-Emodin in mouse plasma. *J. Chromatogr, B.* **796**: 113-119.